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16. ABSTRACT

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THE ORDINATION OF AQUATIC NEMATODE COMMUNITIES AS AFFECTED BY STREAM WATER QUALITY

by
C. A. CALLAHAN, V. R. FERRIS, and J. M. FERRIS

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THE ORDINATION OF AQUATIC NEMATODE COMMUNITIES AS AFFECTED BY STREAM WATER QUALITY[1]

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SUMMARY. Benthic nematodes were sampled at 16 sites on two streams to investigate the relationships of nematode community structure to various water quality factors. A prominence value for each species was calculated for use in three-dimensional community ordination. Species composition of the sites, nematode density, and species interaction with certain physico-chemical factors were significant in determining the pattern, or clustering, of the sites in the ordinations. The sites found in clusters with low densities of all species were those with the lowest level of nutrients (i.e., nitrate and nitrite nitrogen, total phosphate, and dissolved reactive phosphate), and the highest level of dissolved oxygen. The density of nematodes observed at each site was inversely correlated with median size of benthic substrate. When the number of species used in the analysis was decreased from 154 to 6 dominant species, a closer relationship of nematode species with the physico-chemical factors was observed. These six dominant species are most influenced by the physical and chemical parameters measured and they are most important in determining clustering of sites.

KEY WORDS. benthic nematodes, community structure, ordination, water quality.

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1. INTRODUCTION

Chemical and physical conditions are traditionally used to set water quality standards. The relative ease in collection of certain of these data is a distinct advantage. However, deleterious effects of pollutants may be modified by a wide range of concentrations and by numerous other substances and conditions. These additional factors make chemical evaluation more difficult. The use of aquatic organisms to monitor and assess water quality takes advantage of an 'accumulative effect'; i.e., biological organisms reflect the past range of environmental factors whereas chemical and physical parameters generally reflect conditions during the short period of sampling. Also, the sensitivity of biological systems may be such as to indicate harmful effects from toxic substances at very low levels.

Nematodes are ubiquitous, occurring in terrestrial and aquatic habitats as parasites of plants and animals and as free living entities. Although nematodes thrive in habitats with wide ranges of moisture levels, they occur in large numbers in all aquatic environments. Because of wide distribution and abundance, benthic nematode communities should reflect ecological changes that can be quantified. Nematodes however have never been included in biological assessment for ecological change.

Community structure analyses generally are concerned with macroinvertebrates, and occasionally microinvertebrates, with nematodes generally being indicated at the phylum level, if at all. The present study of benthic nematode communities in streams was undertaken to investigate the relationship between nematode communities and various measures of water quality. The objectives were (1) to describe and analyze differences in the community structure of benthic nematodes, (2) to ascribe these differences to different water quality parameters and physical parameters, and (3) to develop a means of detecting ecological changes based on benthic nematode community structure.

2. WATER QUALITY SAMPLING

Two first order streams, Little Pine Creek and Indian Creek, Indiana, minor tributaries of the Wabash River, were chosen for this study (Figure 1). Indian Creek is wholly within Tippecanoe County, whereas Little Pine Creek enters the Wabash River in Warren County. Both creeks flow in a north to south direction transecting Tippecanoe County. Sample sites were chosen on each stream to represent different physiographic habitats, with some consideration also given to accessibility. Eight sites were selected on each stream and were coded consecutively from the origin to the mouth of the stream.

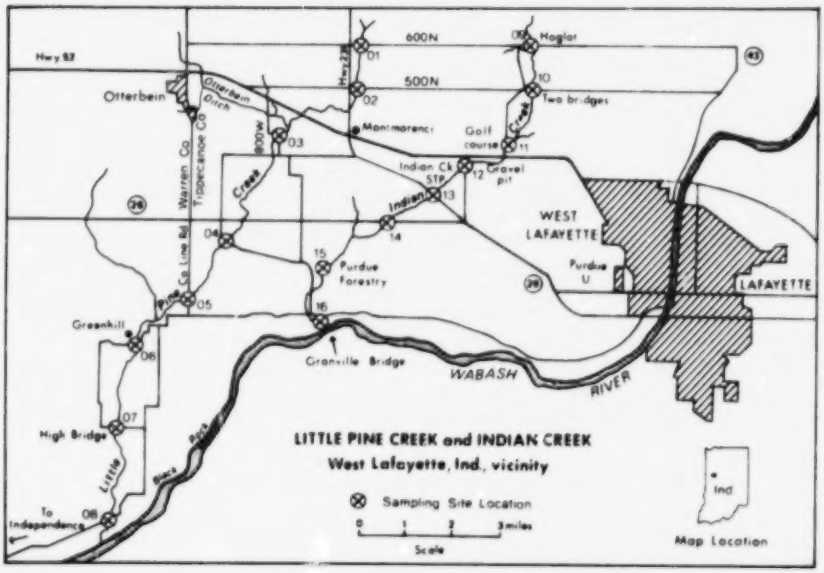

FIG. 1: Sampling site locations on Little Pine Creek and Indian Creek in the vicinity of West Lafayette, Indiana.

Water samples were collected from the 16 sites twice a month during the months of June, July, August, September, and October, and once a month during the months of March, April, May, and December. The order in which the samples were collected was the same on all dates. Measurements of water quality taken included pH, temperature, dissolved oxygen, nitrate-nitrogen, nitrite-nitrogen, methyl orange alkalinity, carbon dioxide, total phosphate, and dissolved reactive phosphate.

Data for statistical analysis were separated for each year for the two streams. A two-factor design was used (streams and sites) with sites nested in streams. Bartlett's test for homogeneity was used to test for homogeneity of variance (Steel and Torrie, 1960). Following this test, the data for nitrate-nitrogen ($N\text{-}NO_3$) and nitrite-nitrogen ($N\text{-}NO_2$) were transformed using a square root transformation to normalize variance.

Seven physico-chemical factors were significantly different between sites (nitrite-nitrogen, nitrate-nitrogen, total phosphate, dissolved reactive phosphate, pH, organic matter, and dissolved oxygen).

3. TECHNIQUES FOR BENTHOS SUBSTRATE CHARACTERIZATION (TEXTURE ANALYSIS)

The substrate at each site was examined by texture analysis, in which relative quantities of particles of different sizes in the sample were determined (Morgans, 1956; Krumbein, 1939). The sites studied exhibit a diversity of substratum types. However, two broad categories can be recognized: those that are predominately silt, clay and fine sand, and those that are composed chiefly of coarse sand and larger particles. Generally, those sites located where the stream is deep and wide with slow moving water, have much smaller particles than those sites where the stream is shallow and narrow, with swifter current. Analysis of stream velocity data and median particle size (Mdϕ) showed a high degree of relationship between factors ($r = 0.65$).

4. BENTHOS CHARACTERIZATION (ORGANIC MATTER)

Organic matter content of benthos samples was estimated by the loss of weight on ignition (Cummins, 1962). There was a trend toward higher values for those sites where the stream is deep and the water slow moving (permitting settling out of suspended material). Factors determining the amount of organic material are (1) available sources of allochthonous material, (2) substrate stability and texture, and (3) stream characteristics such as velocity and depth.

5. AVERAGE STREAM VELOCITY

The average stream velocity at each site was measured three times by recording the time required for a float to travel a prescribed distance. These measurements were averaged and converted to cm/sec (Robins and Crawford, 1954).

6. SAMPLING TECHNIQUES FOR NEMATODES

The 16 sites were sampled for nematodes in April, July, and October during 1970 and 1971. At each collection time, duplicate samples were collected from the stream bottom on the same side of the stream at each site using a small scoop. One half of the nematode material collected was saved for counting. Counts were

made at 35X magnification on a compound microscope using a counting chamber such that small specimens could be examined at 100X magnification.

The nematodes were processed to glycerine, sorted, and mounted on Cobb aluminum slides. A camera lucida sketch was prepared for each species found, measurements recorded, and the literature searched for species identification. All species, including those which could not be identified from the literature, were given a species code letter which was used throughout the study. Approximately 4,500 specimens on slides were examined.

A total of 154 species of nematodes representing nine orders were found during the two-year period of this study. Members of the order Dorylaimida were the most numerous in genera (29) and species (55), whereas the order Tylenchida was second most numerous in genera (15) and species (36). The remaining orders contained from one to nine genera. The total number of species found at each site over the two-year period ranged from a low of 28 (site 7) to a high of 97 (site 3). An average of 17 species per sample were found for all sites per sample time.

Slides of all specimens used in the study have been placed in the Purdue Nematode Collection, Department of Entomology, Lafayette, Indiana. All sketches and measurements prepared are a part of the catalogues of that collection.

7. CALCULATION OF PROMINENCE VALUES AND ORDINATION TECHNIQUE

A prominence value for use in the three dimensional community ordination technique (Bray and Curtis, 1957) was calculated for each species at each site, by multiplying the species density by the square root of the relative frequency (Beals, 1960; Callahan, 1976). The computer program ORDCOM, written by Dr. R. L. Giese formerly of the Purdue Department of Entomology, was used for the analysis. In this technique, sites are compared on the basis of their dissimilarities in nematode population structure. Coefficients of similarity-dissimilarity are calculated as:

$$C = 2w/a + b,$$

where

a = the sum of the prominence values of all species in one site.

b = sum of prominence values of all species in another site.

w = sum of prominence values for each species the two sites have in common; the prominence value selected is the lowest for the species common to both sites.

Interpoint distances are calculated from the matrix of coefficients and plotted in a 3-dimensional ordination.

Spearman rank-order correlation coefficients (Downie and Heath, 1970) were calculated for several statistics related to nematode community structure, and several physico-chemical characteristics of the stream sites. The nematode statistics used were the number of dominant species (those with prominence values greater than 100), total number of nematodes, and the number of species (Table 1). The physico-chemical characteristics tested were stream velocity, organic matter, total and dissolved reactive phosphate, nitrite-nitrogen, nitrate-nitrogen, and median particle size of the benthic substrate.

8. COMMUNITY ORDINATION AND COMMUNITY STRUCTURE ANALYSIS

The comparative ordination technique (Bray and Curtis, 1957) is among the many mathematical methods used to summarize community structure (Thomas, 1972). The technique arranges quantitative data in multi-dimensional order to arrive at an objective classification of ecological associations. Importance values derived from the density and frequency of individual species form the basis for the ordination technique (Beals, 1960; Johnson *et al.*, 1973; Callahan, 1976).

8.1 Year to Year Comparisons. Results of the community ordination based on species prominence values for 1970 and 1971 for sites on both streams are presented in three planes (Figure 2). Points 1 and 9 are the same location (e.g. site 1), point 1 representing data from 1970 and point 9 representing data from 1971; point 2 representing data from 1970 and point 10 representing data from 1971, and so on. Since the position of a site based on 1970 data is very close to the position of that same site for 1971 data, these show good reproducibility of data between years. Points 1 and 9 (1970 and 1971 data for site 9), and 3 and 11 (site 11) and 8 and 16 (site 16) are generally closer to each other than to paired points of other sites (Figure 2).

Two general patterns are evident in the clustering of the nematode species observed at the individual stream sites: (1) usually the sites and their counterparts are very closely positioned in all planes of the ordinations and (2) groups formed by these sites remain closely associated throughout all planes. This

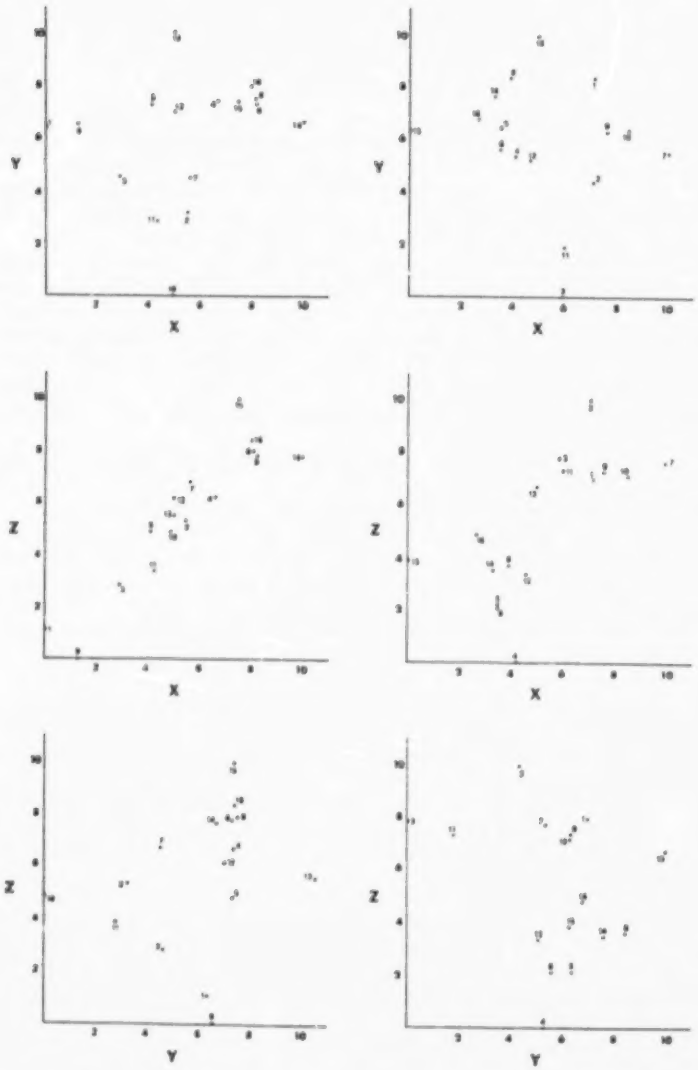

FIG. 2: Ordination in X-Y, X-Z, *and* Y-Z *planes of eight sites on Indian Creek (column* 1*) and on Little Pine Creek (column* 2*) based on* 154 *nematode species. Points* 1-8 *represent data from the year* 1970 *and points* 9-16 *represent data from* 1971.

pattern or strong association of sites observed between the two separate years is due to strong site influence.

8.2. Total Data Comparisons. The first column of Figure 3 shows the results of community ordination based on species prominence values computed by combining the data from both years (1970 and 1971) for each stream and designating the sites on Little Pine Creek (LPC) numbers 1 through 8 and the sites on Indian Creek (IC) numbers 9 through 16. Since separate ordinations of these data (Figure 2) from each stream indicated that differences between years were small, it seemed reasonable to combine the data (both years and streams for a broader outlook. The analysis of the total data by ordination permits a comparison of many nematode communities existing in many types of environments, each with a range of physico-chemical characteristics, to arrive at a better understanding of the factors affecting these nematode communities.

Three distinct clusters of sites were observed in all planes (3, 10, 11); (1, 2, 7, 9); and (4, 5, 6, 8, 12, 13, 14, 15, 16) when data from both years were combined and ordination performed on mean values for both years, 1970 and 1971. The clustering of sites was influenced by the same factors and followed a similar pattern for total data as was observed for LPC and IC independently. That is, those sites on each stream with similar species, high densities, and similar site characteristics were clustered together.

Sites 1, 2, 7, and 9 are clustered together on the low x and the high y axis (Figure 3). These sites have high numbers of *Monhystera* A and B and *Mononchus* A with relatively low numbers of *Mesodorylaimus*, *Tylenchus*, and *Acrobeloides*. Sites 1, 2, and 7 clustered together on LPC and were influenced in that ordination, as in this one, by density of nematodes. Site 9, from IC, also had a high density of nematodes. All of these sites have very large numbers of *Monhystera* B.

Sites 3, 10, and 11, once grouped with other high density sites on their respective streams (Figure 2), are now clustered together in a lower y axis and the medium x axis (Figure 3). These three sites have high numbers of all six species except *Monhystera* A and B which are present in moderately low numbers (Table 1). Note that the position of site 11 is near the cluster of sites 1, 3, 7, and 9. This is due to the large number of *Monhystera* B present at this site. Site 11 is in close proximity to these four sites.

The remainder of the sites are grouped together on the high x and high y axis which is consistent with the groupings observed

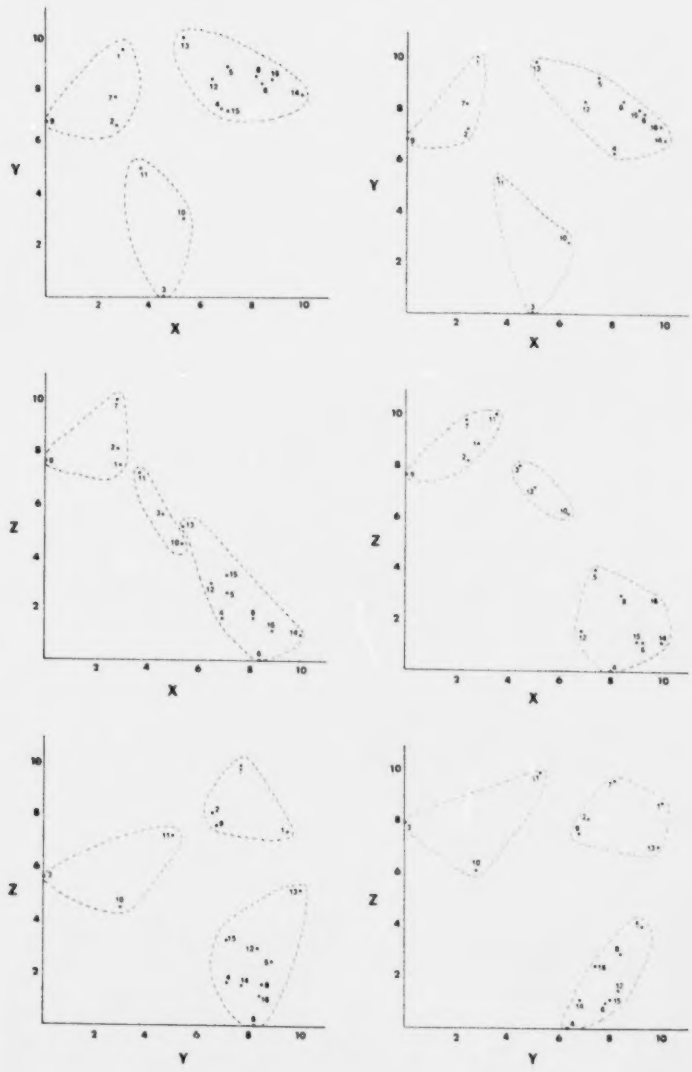

FIG. 3: Ordination in X-Y, X-Z, *and* Y-Z *planes of* 16 *stream sites (sites* 1-8, *Little Pine Creek sites* 9-16, *Indian Creek) based on* 154 *species for the two year period* 1970-1971 *(Column* 1*) and on six dominant nematode species for the two year period (Column* 2*).*

TABLE 1: The total number of the six dominant species observed at all sites, both years (total data).

Site	*Mesodorylaimus* #4	*Tylenchus*	*Acrobeloides*	*Monhystera* A	*Monhystera* B	*Mononchus*
1	82	351	133	4,770	14,220	253
2	2,990	2,181	397	5,846	15,911	2,430
3	30,159	5,683	3,750	376	2,372	2,375
4	1,587	1,544	43	103	627	217
5	132	410	52	391	3,599	64
6	102	605	272	115	1,285	192
7	121	537	11,509	7,023	14,488	47
8	147	73	62	742	1,798	280
9	1,484	508	449	28,743	41,490	1,285
10	8,952	4,019	1,986	231	958	1,402
11	6,291	2,964	5,117	770	11,156	10,592
12	584	977	776	1,191	103	234
13	292	1,004	1,778	959	7,860	428
14	88	371	41	31	841	1,024
15	115	522	221	7	1,691	234
16	41	362	29	20	1,609	911

for the individual streams. Also, this group did not change in relative position when comparing the position of these sites in the IC ordination, but LPC sites 4, 5, 6, and 8 apparently have shifted upward on the x axis.

Species composition and density are very important in determining the pattern or clustering of the sites in the ordinations. The clustering of sites was shown to be influenced by six dominant species, *Monhystera* A and B, *Mesodorylaimus* #4, *Monochus* A, *Tylenchus* A, and *Acrobeloides* sp. IC sites with higher densities were grouped nearer the origin of the axes whereas LPC sites with high densities were grouped toward the extremities of the axes.

The 'total data' analysis shows that sites with the highest densities and most similar species composition, of the six species, were grouped together. Other sites, with lesser numbers of these six species or when one or more were absent, were grouped in a cluster that was removed from the high density sites.

Figure 3 shows results of an ordination analysis of the same 16 sites used for total data analysis using only the six predominant species listed in Table 1. The three clusters shown in the second column of Figure 3 are essentially the same as those seen in the first column, with some minor shifts in sites 11 and 13. Apparently with fewer species the association of sites 11 and 13 to sites 1, 2, 7, and 9 is intensified.

9. DISCUSSION

Species composition and numbers of individuals found at the sites determined the pattern or clustering of the sites in the ordinations. The clustering of the sites was shown to be influenced by six of the dominant species. *Monhystera* A and B, *Mesodorylaimus* #4, *Tylenchus* A, *Mononchus* A and *Acrobeloides* sp. By decreasing the number of species from 154 to 6, the correlations between data related to the nematode community and data relating to physico-chemical factors were enhanced (Table 2). It must be assumed that these six species are most influenced by the physical and chemical factors tested and these species are most important in determining species similarity between sites. When species of lesser importance are removed from consideration in the ordination correlations between certain species and physico-chemical characteristics become more pronounced. The sites found in clusters with low densities were the sites with lowest level of nutrient (i.e., nitrite and nitrate-nitrogen, ortho and total phosphorus), and the highest level of dissolved oxygen. These sites represent the sites with a lower level of disturbance.

TABLE 2: Results of Spearman's rank-order correlation (Downie and Heath, 1970) for certain physico-chemical factors comparing rankings of all species to rankings of six of the dominant species.

Factor	Ranking order of sites	Spearman's rho(r) All species	Six dominant
Mdϕ	large to small*	r = .476	r = .769
Stream velocity	low to high	r = .376	r = .485
Nitrate-nitrogen	high to low	r = .221	r = .472
Nitrite-nitrogen	high to low	r = .529	r = .471
pH	low to high	r = .488	r = .704
Organic matter	high to low	r = .382	r = .503

* large Mdϕ = small median particle size.

The species present and their numbers at any given time seem to be the result of an interplay between a number of physical, chemical, and biological characteristics observed at the stream sites.

Distinct differences in the amounts of various size classes of benthic material observed between sites could account, in part, for the differences or similarity, as the case may be, in nematode community structure, thus influencing the clustering of sites. The density of nematodes observed at each site was found to be correlated ($r = .5$, $P = .05\%$) with benthic substrate size. That is, larger numbers of nematodes were found at sites with smaller median benthic particles and sites with low densities of nematodes were sited with large median particle size.

Those sites with benthic material of small median diameter were in areas of the streams that generally experienced slow velocities and had relatively deep pools (sites 3, 10, and 11). Baliga *et al.*, (1969) assumed that the pooled areas were most conducive to nematode population buildup and propagation due to the lack of disturbance by fast stream velocities. He assumed that the primary source of nematodes was from sewage treatment plants via the effluent discharge reaching the stream and the greatest buildup occurred below the outfall. Unlike Baliga's study, nematode densities in these two streams were observed to be comparable in areas above and below sewage treatment plant outfalls. In fact, density of species was correlated with median particle size (as influenced by stream velocity) as well as nutrient enrichment.

It appears that nematode buildup and the number of species observed at the sites are related to nutrient enrichment and the median particle size of the benthic material. Total numbers of

nematodes were significantly correlated to nitrite-nitrogen levels ($r = .5$, $P = .1\%$) and the number of species at the sites was correlated with the median particle size ($r = .7$, $P = .05\%$).

Those sites observed to have the highest level of nutrients also had the largest number of nematodes and the largest number of the six dominant species. Chemically enriched areas of streams often support an increased number of variety of species. Egglishaw and Morgan (1965) reported that with few exceptions they observed a greater mean number of all groups and main species of animals under such conditions. MacKay (1969) reported similar results when she concluded that the most "important" communities are found in these areas of the stream. Although several of the sites on these streams have both high nematode populations and high levels of organic material, no definite correlations exist. These data do suggest that where increased organic matter associated with slow currents (as found in pooled areas) and a high level of nitrite-nitrogen are found, the total number of nematodes as well as the number of species will be very high compared to sites lacking this combination.

Although nitrate-nitrogen is the usual form of nitrogen found in water, Hynes (1970) reported that large amounts of nitrite-nitrogen generally indicates gross pollution sources. Nelson (1972) indicated that denitrification may be significant pathway for the removal of nitrates from rivers and ponds. Chen *et al.*, (1972) also indicated denitrification to take place in sediments with the accumulation of nitrite-nitrogen at measurable levels.

The nitrite-nitrogen levels observed in these two streams gradually decrease with stream length, as expected, indicating a rapid uptake by plants. The distribution of nitrite-nitrogen concentration for these streams follows the same pattern as seen for potential large inputs were very close to sewage treatment outfalls (sites 3 and 13) and open feedlots (site 9) where animals were permitted direct access to the stream.

Some studies (Butcher, 1946; Patrick, 1964, 1971) have concluded that disruption of aquatic communities (by gross population) causes a reduction in the total number of species while individuals of certain species become more abundant. Patrick (1972) reported that the first effect of an increase in nutrients such as nitrogen and phosphorus is to cause certain species to become more common. With higher nutrient levels, more significant species changes take place, resulting in the elimination of species. The density of nematodes as well as the number of species at the stream sites were shown to increase with increased nutrient levels. Sites on the streams considered to be enriched on perturbed are those

areas where high nutrient levels were observed (sites 1, 2, 3, 9, 10, 11, and 13). Many of these sites had high densities and large numbers of species.

Although the range of nutrient concentrations was great enough at the stream sites to produce discernable patterns in community structure, the range did not produce a significant increase in numbers per species nor a significant change in species that would be expected under conditions of high nutrient loading (i.e., gross pollution). This study indicates that benthic nematode communities at the stream sites were influenced by an interplay of physical and chemical factors observed.

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INTERNATIONAL STATISTICAL ECOLOGY PROGRAM

The International Statistical Ecology Program (ISEP) consists of the activities of the Statistical Ecology Section of the International Association for Ecology and of the Liaison Committee on Statistical Ecology of the International Statistical Institute, the Biometric Society, and the International Association for Ecology. The ISEP is a non-profit program formulated to serve the needs of interdisciplinary research and training in the newly emerging fields of Statistical Ecology and Ecological Statistics.

SATELLITE PROGRAM IN STATISTICAL ECOLOGY

The Second International Congress of Ecology was held in Jerusalem during September 1978. In this connection, ISEP organized a Satellite Program in Statistical Ecology during 1977 and 1978. The emphasis was on research, review, and exposition concerned with the interface between quantitative ecology and relevant quantitative methods. Both theory and application of ecology and ecometrics received attention. The Satellite Program consisted of instructional coursework, seminar series, thematic research conferences, and collaborative research workshops.

Research papers and research-review-expositions were specially prepared for the program by concerned experts and expositors. These materials have been refereed and revised, and are now available in a series of ten edited volumes listed on page ii of this volume.

The Satellite Program takes as its theme the better melding of fundamental ecological concepts with rigorous empirical quantification. The overall result should be progress toward a stronger body of general ecologic and ecometric theory and practice.

FUTURE DIRECTIONS

The satellite-like-programs help create and sustain enthusiasm, inward strength, and working efficiency of those who desire to meet a contemporary social need in the form of some interdisciplinary work. It should be only proper and rewarding for everyone involved that such programs are planned from time to time.

Plans are being made for a satellite program in conjunction with the next Biennial Conference of the International Statistical Institute and the next International Congress of Ecology. Care should be exercised that the next program not become a mere replica of the present one, however successful it has been. Instead, the next program should be organized so that it helps further the evolution of statistical ecology as a productive field.

The next program is being discussed in terms of subject area groups. Each subject group is to have a coordinator assisted by small committees, such as a program committee, a research committee, an annual review committee, a journal committee, and an education committee. This approach is expected to respond to the need for a journal on statistical ecology, and also to the need of bringing out well planned annual review volumes. The education committee would formulate plans for timely modules and monographs. Interested readers may feel free to communicate their ideas and interests to those involved in planning the next program. The mailing address is: International Statistical Ecology Program, P. O. Box 218, State College, PA 16801, USA.

International Statistical Ecology Program
Satellite Program in Statistical Ecology

CONTENTS OF EDITED VOLUMES

BACKGROUND

Close to 350 lectures and discussions were held during 50 days in the middle of the summer seasons of 1977 and 1978. The program was endowed with stimulating local setting as well as with atmosphere conducive for scholarship. Participants represented broad background and expertise. Advice was sought from a number of special advisors. The advice received was immensely helpful in guiding selection of the best experts in the field to achieve as representative and balanced a coverage as possible.

The editors together with the referees took a rather critical and constructive attitude from initial to final stages of preparation of papers by offering specific suggestions concerning the suitability, and also the structure, content, and size. These efforts of coordination and revision were intensified through editorial sessions at the program itself as a necessary step for the benefit of both the readers and the participants. Everyone went by scientific interests often at the expense of personal preferences. The program atmosphere became truly creative and friendly, and this remarkable development contributed to the maximal cohesion of the program and its proceedings within the limited time period available.

The edited research papers and research-review-expositions prepared for the program are organized in ten volumes. Altogether, they consist of an estimated 4,000 pages of research, review, and exposition in addition to a common foreword in each, followed by individual volume introductions. Subject and author indexes are also prepared for each volume. May this ten volume set serve as a useful stimulus to further research and training in statistical ecology for the nineteen eighties.

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